

## **Activities Associated with the NTP High Throughput Screening Initiative**

The NTP Roadmap for the 21st Century includes a major initiative to develop high throughput screening (HTS) assays to:

- identify mechanisms of action for further investigation
- develop predictive models for biological response
- prioritize substances for further toxicological evaluation

In support of this initiative, the NTP became a formal participant in the NIH Molecular Libraries Initiative (MLI) in July 2005. The MLI – a part of the NIH Roadmap for Medical Research – is focusing on the use of high-technology screening methods to identify small molecules that can be optimized as chemical probes to study the functions of genes, cells, and biochemical pathways. The NTP, through its association with the MLI, has the opportunity to generate information that links data on the biological activity of substances generated from biochemical and cell-based HTS assays with toxicity endpoints identified in the NTP's toxicology testing program. To achieve this goal, the NTP began a formal collaboration in August 2005 with the NIH Chemical Genomics Center (NCGC) to test substances of interest to the NTP across a spectrum of HTS assays. The NCGC is one of 10 HTS centers around the country that comprise the NIH Molecular Libraries Screening Center Network (MLSCN).

Late in 2005, the NTP supplied six cell-based assays from Promega Corporation to the NCGC. These assays included two for cytotoxicity (CellTiter-Glo which measures ATP levels and Cytotox-One which measures LDH release), three for specific caspase enzyme activity associated with apoptosis (Caspase 3/7, Caspase 8, and Caspase 9), and one for activity associated with a g-glycoprotein pump located in the cell membrane responsible for drug resistance (pgp-Glo Assay). These assays were selected largely because it appeared that they could easily be optimized for use in the 1536-well robotics assay format used by the NCGC, because the endpoints could be of interest from a toxicological viewpoint, and because it was thought that the data generated by these tests could be used to establish bioinformatic procedures needed to appropriately mine the extraordinary large sets of chemical and toxicological data that would result from the NTP HTS initiative.

In January 2006, the NTP completed the shipment of a total of 1408 test substances to the NCGC (based on the plate design used by the NCGC, 1408 represents the number of chemicals that are tested per 1536-well plate). These substances were selected because they have been tested by the NTP in their bioassay program and some amount of toxicity data exists for them in the NTP database and/or because they represented reference substances for toxicological endpoints of interest (e.g., dermal corrosion, acute toxicity, endocrine activity).

These substances were shipped in individual polypropylene vials in racks of 96 vials per rack. The vials had barcodes on the bottom and spreadsheets of the contents and position of each vial on the rack was sent with each shipment. The vials contained 1.0 mL of a 10 mM solution of the test substance in DMSO, as required by NCGC.

The list of test substances sent to the NCGC includes nearly every chemical class for small molecules imaginable. Molecular weights ranged from approximately 100 to 400. The list

consists of substances used as solvents, fire retardants, preservatives, flavoring agents, plasticizers, therapeutic agents, pesticides and natural products as well as inorganic and organic pollutants and drinking water disinfection byproducts.

To date, the NTP collection of 1408 chemicals has been tested in the CellTiter-Glo cytotoxicity assay using eight human and three rodent cell lines/strains/primary cells. The eight human cell lines/strains are:

- BJ (transformed foreskin fibroblasts)
- HEK293 (human embryonic kidney)
- HepG2 (human hepatocellular carcinoma)
- HUV-EC-C (normal endothelial cells)
- Jurkat (Clone E6-1, human T cell leukemia)
- MRC-5 [lung fibroblast cell strain)
- SH-SY5Y (human neuroblastoma)
- SK-N-SH (human neuroblastoma)

The six rodent cell lines/primary cells are

- buffy coat (rat leukocytes)
- H-4-II-E (rat hepatoma)
- N2A (mouse neuroblastoma)
- NIH 3T3 cells (mouse transformed fibroblasts)
- renal proximal tubule cells (rat primary epithelial cells)
- SVEC4-10EHR1 (mouse transformed epithelial cells)

Normalized data from six of the human cell types have been placed on the publicly accessible PubChem database, maintained by the National Library of Medicine; the remainder of the data should be placed on PubChem by the end of this year. Raw and normalized data have been made available to NTP for internal analysis.

In addition, the NTP 1408 have been tested for caspase-3/7 activity in five human cell types and three rodent cell types, and the Cytotox-One LDH release assay has been standardized for one human and one rodent cell type.

Also, in December 2005, a collaboration was established between the NTP HTS Faculty and the EPA Chemical Prioritization Community of Practice (CPCP) to jointly evaluate HTS assays and other model systems for their use in toxicological investigations and in chemical prioritization.

In February 2006, three joint NTP-EPA working groups were established:

- Toxicity Targets and Bioactivity Assays, the purpose of which is to identify toxicity targets and bioactivity assays targets of interest relating to various toxicities, and relevant in silico, HTS, and high content screening assays
- Chemical Selection, the purpose of which is to identify and coordinate the testing of chemicals of interest to the NTP and the EPA
- Informatics, the purpose of which is to develop and/or identify appropriate tools for chemical and biological data analysis and management across multiple databases.

To broaden the exposure to HTS concepts as they apply to toxicology testing within the NIEHS/NTP and its counterpart at the EPA (Chemical Prioritization Community of Practice), NTP hosted the directors of the NCGC in a day-long Chemical Genomics 101 course, held at NIEHS in RTP on April 20, 2006. Approximately 100 scientists from NIEHS, EPA, and non-government organizations attended.

In collaboration with the EPA, the NCGC, and Pharma, the NTP is organizing a special session at the 2007 annual meeting of the Society for Biomolecular Sciences entitled “Toxicity Profiling using High-Throughput and High-Content Technologies”. The purpose of this session is to discuss the challenges associated with the use of HTS and high content screening assays to identify activities of diverse chemical compounds in toxicity-relevant assays, to facilitate the development of predictive *in vitro* models for toxicity, and to help prioritize substances for further toxicological evaluation.